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***Ailanthus altissima* Aqueous Extract Deters *Spodoptera frugiperda* Oviposition**

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Abstract

Ailanthus altissima (Mill.) Swingle (Simaroubaceae), the tree of heaven, is a highly invasive tree species containing phytochemicals with a range of biological activities. Exposure to novel chemistry from invasive plant species may result in changes to established Lepidopteran behaviors, including feeding or oviposition. However, the impact of *A. altissima* chemistry on insect behavior has not been extensively explored. Therefore, *A. altissima* extract was tested for oviposition deterrence effects against *Spodoptera frugiperda* (J. E. Smith) (Noctuidae). Oviposition substrates were treated with water-soluble extractions of *Zea mays* (L), *A. altissima*, or a combination of both. Moths were then placed in chambers containing different substrate treatments and allowed to oviposit. Given choice, *S. frugiperda* demonstrated a higher percentage of egg deposition on *Z. mays* treated substrate compared to water treated substrate or *A. altissima* treated substrate. Significantly, when *Z. mays* treated substrate was subsequently treated with *A. altissima* extract, *S. frugiperda* was deterred from laying eggs on its preferred substrate (*Z. mays*) in a concentration-dependent manner. This observed change in behavior suggests that the deterrent properties of *A. altissima* phytochemicals may have economically important crop protection applications in controlling pest species like *S. frugiperda*.

Keywords: Oviposition, phytochemicals, behavioral deterrent, *Ailanthus*

Phytochemicals embedded in the leaf cuticle or within leaf tissues are known to play significant roles in plant host selection by Lepidoptera (Thompson and Pellmyr 1991, Renwick and Chew 1994). Host selection can be based on the presence of unique phytochemistry (Haribal et al. 1996), the concentration of select phytochemicals (Pereyra and Bowers 1988), or the relative proportions of different compounds within the tissue (Nishida et al. 1987). Host specific cues can positively influence behavior as seen by Meagher et al. (2011) where *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) preferentially selected grass when given a choice between corn, forage grass, and an artificial surface. Ultimately, the process of host selection for oviposition is relatively complex, potentially integrating multiple signals, to result in a behavioral choice (Lund et al. 2019).

With the establishment of invasive plant species into local ecosystems, opportunities exist to explore the impact of novel phytochemicals from these plants (Cappuccino and Arnason 2006) on oviposition. *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae), the tree of heaven, is a highly successful invasive tree species (Mastelić and

Jerković 2002) containing phytochemicals with a range of biological activities (Alves et al. 2014). Methylene chloride extracts from *A. altissima* leaves inhibited germination and growth of alfalfa, *Medicago sativa* L. (Fabaceae), and showed weak insecticidal properties against *Aedes aegypti* L. (Culicidae) (Tsao et al. 2002), while Pavela et al. (2014) demonstrated antifeedant activity in *Spodoptera littoralis* (Boisduval) associated with *A. altissima* methanol extracts. The potential use of novel phytochemicals from invasive plants, like *A. altissima*, to deter oviposition prior to host selection represents an exciting application for economically valuable crops.

One of the most widespread and damaging insect pests to cash crops is the fall armyworm, *S. frugiperda*, which feeds primarily on plants in the grass family (Nagoshi et al. 2012) with its most severe impact on corn, *Zea mays* L. (Poaceae) (Cruz et al. 1999). To reduce the damage caused by the fall armyworm and other crop pests, the United States spends over \$10 billion annually on synthetic pesticides that, when applied, cause an increase in acute poisoning; cancer, and chronic diseases in humans; contaminated food products; destruction of

beneficial predators; pesticide resistance; honey bee poisoning; and crop damage (Pimentel 2005). Therefore, the development of alternative management resources is of great interest. The purpose of this research was to evaluate the biological properties of *A. altissima* with the specific objective of determining if its phytochemicals can influence Lepidopteran oviposition behavior.

Spodoptera frugiperda larvae were purchased from Benzon Research (Carlisle, PA) and reared at 21 °C with 16:8 h L:D cycles until pupation. Pupae were transferred by hand to individual 100 x 15 mm petri dishes until emergence. Newly emerged moths were kept isolated for two days to optimize fecundity (Rogers and Marti 1994).

Ailanthus altissima leaflets were collected from the campus of Millersville University and stored at -20°C. Four-week-old *Z. mays* was grown from seed in the Millersville University greenhouse (25°C, 14:10 h L:D cycles) and harvested immediately prior to extraction. Tissue (25 g) was pulverized with mortar and pestle then extracted with deionized, distilled water (100 ml) for one hour. The supernatant was clarified by centrifugation (5 min, 2,400 rpm, 21°C) and applied to oviposition substrates.

Twenty-six oviposition chambers were constructed using 950 ml mason jars containing cotton balls soaked in 10% sucrose as the moth food source. Cotton balls were replaced every 48 hours. Strips of chromatography paper (Whatman #1), 12 x 4 cm long, were saturated with their respective treatment, air dried for 2 h, and suspended into the chambers. Undiluted *A. altissima* extract contained 13.6 µg/ml residue with a final application concentration (1.0x) of 10.2 mg/strip. Control strips were treated with deionized water only. Each oviposition chamber represented one replicate.

Five male and five female *S. frugiperda* were introduced into each of thirteen oviposition chambers containing four different oviposition substrate treatments including: deionized water (control), *Z. mays* only, *A. altissima* only, and *Z. mays*/*A. altissima* combination. The *Z. mays*/*A. altissima* combination was prepared by sequentially treating the substrate in *Z. mays* extract, followed by *A. altissima* extract, with drying periods (2h) after each application. Thirteen chambers were prepared (as described) to test a concentration series whereby *A. altissima* extract concentrations of 0.0x, 0.25x, 0.5x, and 1.0x were applied onto substrates previously treated with *Z. mays* extract. The 1.0x concentration consisted of undiluted extract.

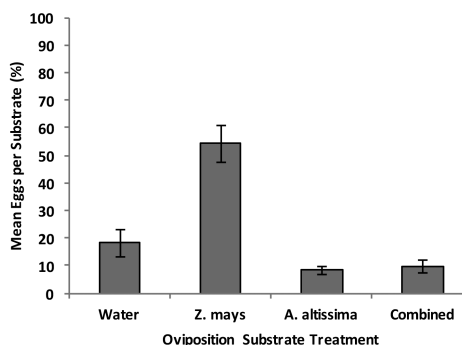


Figure 1. Mean (\pm SE) percentage of eggs laid by *S. frugiperda* after five days on oviposition substrates in a choice experiment. Oviposition substrates were prepared with four treatments including, water only, *Z. mays* extract only, *A. altissima* extract only, or *Z. mays* + *A. altissima* extract (combined) ($F = 18.33$; $df = 3, 48$; $P < .0001$).

After 5 days, substrates were collected and the eggs on each strip were counted and recorded. Eggs masses were examined by gently teasing apart egg mass layers to reveal individual eggs using an Olympus SZ3060 stereo microscope. Percent data were arcsin transformed and normality determined using an Anderson-Darling test. Differences in egg deposition were analyzed using one-way ANOVA and Tukey HSD post hoc test. Statistical analyses were completed using Microsoft Excel® for Mac 2011 version 14.6.6.

A total of 9,220 eggs were found on oviposition strips across 13 oviposition chambers examining host preference (Fig. 1). *Spodoptera frugiperda* demonstrated significantly more egg deposition on substrate coated with *Z. mays* extract ($\bar{x} = 54.2\%$) compared to water ($\bar{x} = 18.3\%$) or *A. altissima* extract ($\bar{x} = 8.3\%$) ($F = 18.33$; $df = 3, 48$; $P < .0001$) (Fig. 1). *Zea mays* extract (alone) was preferred 6.5x more than the *A. altissima* extract (alone) and 5.5x more than the combined *Z. mays* + *A. altissima* extracts ($\bar{x} = 9.7\%$) (Fig. 1). A post hoc Tukey test showed no significant difference in oviposition activity exists when comparing the negative control (water) with *Z. mays* + *A. altissima* extract or *A. altissima* extract alone (Fig. 1).

When the two extracts were combined, egg deposition on *Z. mays* substrates decreased as the concentration of *A. altissima* extract increased ($F = 31.69$; $df = 3, 48$; $P < 0.001$) (Fig. 2). A post hoc Tukey test showed differences exist between 0.0x ($\bar{x} = 46.9\%$) and 0.25x ($\bar{x} = 25.6\%$) as well as 0.25x and 0.5x ($\bar{x} = 12.3\%$); no significant difference was present between the 0.5x and 1.0x ($\bar{x} =$

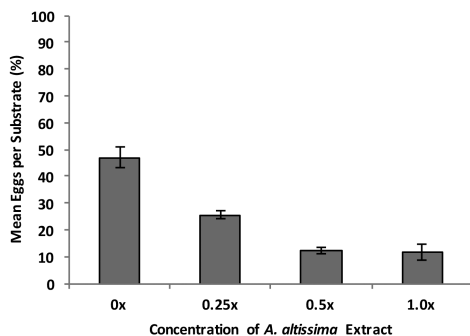


Figure 2. Mean (\pm SE) percentage of eggs laid by *S. frugiperda* on oviposition substrates treated with varying concentrations (0x, 0.25x, 0.5x, 1.0x) of *A. altissima* extract ($F = 31.69$; $df = 3, 48$; $P < .0001$). All oviposition substrates were saturated in *Z. mays* substrate and dried prior to application of *A. altissima* extract.

11.6%) concentrations. A total of 7,522 eggs were found on oviposition strips across the 13 oviposition chambers examining dose response (Fig. 2).

In this study, we demonstrated that the aqueous fraction of *A. altissima* contains phytochemicals capable of deterring *S. frugiperda* oviposition in a concentration-dependent manner, when applied to the preferred host substrate (Fig. 2). While it is possible that sequential treatment of the substrate diluted host phytochemical concentration, this is not anticipated as the twice saturated 0.0x control remained attractive to *S. frugiperda*. The decreased frequency of oviposition on *Z. mays* substrate in response to increasing concentrations of *A. altissima* extract suggests that the novel phytochemicals can directly deter oviposition, as seen in Senrunga et al. (2014).

As a nocturnal ovipositor, *S. frugiperda* would not rely on visual cues; instead, volatile, tactile, or chemical contact cues would be more influential on oviposition behavior with tactile cues having been demonstrated to be highly important (Rojas et al. 2003). Interestingly, Rojas et al. (2003) reported that extracts of corn exhibited oviposition deterrent properties, while here we observed a preference for oviposition on corn-treated substrate. This difference in behavior may be due to the presence of different chemical combinations present in the water-soluble fraction here compared to the methanol and hexane fractions used by Rojas et al. (Pandey and Tripathi 2014).

Little is currently known regarding how manipulation of chemical metabolite profiles at sites of oviposition influences

S. frugiperda behavior. While allelopathic activity by *A. altissima* has been extensively investigated (Heisey 1996, Heisey and Heisey 2003, Albouchi et al. 2013), the impact of its phytochemicals on oviposition behavior is less well understood. Through this work, evidence has been provided of previously unknown biological activity present in phytochemicals from *A. altissima* that detrimentally affects *S. frugiperda* oviposition behavior. Extracts from *A. altissima* have been demonstrated to reduce survivorship in pea aphid, *Acyrtosiphon pisum* (Harris) (De Feo et al. 2009) and reduce feeding activity/growth in *S. littoralis* (Pavela et al. 2014). Both De Feo et al. (2009) and Pavela et al. (2014) identified the quassinoid ailanthone as the biologically relevant phytochemical in *A. altissima*, however, neither group addressed oviposition behavior. While ailanthone is potentially present in the extract evaluated in this study, it was not chemically confirmed and it is not clear if this phytochemical could influence oviposition; thus further experiments on this metabolite are warranted.

Our demonstration of reduced oviposition is fundamental to the potential use of *A. altissima* as an alternative oviposition deterrent in an agricultural setting. Overuse of established pesticides has already led to resistance in Noctuidae agricultural pests, including *Spodoptera exigua* (Hübner) (Ahmad et al. 2018) and *Spodoptera litura* (F.) (Shad et al. 2012), emphasizing the need for new crop protection resources. Precedent clearly exists for phytochemical application (Isman 2006) as demonstrated by cypermethrin and permethrin, which both act as effective insecticidal and deterrent agents for *S. frugiperda* (Usmani and Knowles 2001). For example, crude hexane extracts (1% conc.) from curry leaf, *Murraya koenigii* (L.), reduced oviposition in the Noctuid *S. litura* by ~60% (Senrunga et al. 2014). Further, essential oils present in cinnamon, clove, ginger, mint, and thyme significantly reduced oviposition (>80%) by the velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner) when applied to host plants (Ribeiro et al. 2015). To our knowledge, this study represents the first evidence of *S. frugiperda* oviposition deterrence facilitated by phytochemicals from *A. altissima*. Future efforts to identify/isolate the semiochemical(s) present in *A. altissima* will potentially facilitate application in agricultural settings as a replacement for, or in conjunction with standard pesticides.

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